Shamblott and Gearhart

Application No.: 09/767,421 Filed: January 22, 2001

Page 2

PATENT Attorney Docket No.: JHU1750-1

Amendments to the Claims

Please amend claims 1, 9-13, 15-16 and 22-32 as indicated in the listing of claims.

Please add new claims 34-38 as shown in the listing of claims.

Please cancel claim 33 without prejudice or disclaimer.

Claims 2-8, 14 and 17-21 were previously canceled, and claim 33 was previously withdrawn.

The listing of claims will replace prior version and listings of claims in the application.

Listing of Claims:

1. (Currently amended) An enriched human embryoid body derived (EBD) cell population of cells comprising, characterized by:

human embryoid body derived (EBD) cells derived from a human embryoid body, wherein the embryoid body is derived from embryonic germ cells, which are further derived from primordial germ cells not having teratogenic properties in SCID mice, and wherein the EBD cells proliferate in culture upon enzymatic disaggregation from the embryoid body and express at least a first and a second polypeptide or mRNA marker from at least two different cell types, wherein the cell types are selected from ectodermal cells, mesodermal cells, or endodermal cells, and wherein the first marker is selected from the group consisting of nestin, vimentin, neurofilament light isoform, microtubule associated protein 2e, tau, nonphosphorylated neurofilament heavy isoform, neuron specific enolase, tyrosine hydroxylase, glial fibrillary acidic protein, CNPase, and galactocerebroside, and the second marker is selected from the group consisting of myf-6, myosin light chain 2 ventricular isoform, flk1, α -1-fetoprotein, and GATA-4.

2-8. (Canceled).

Shamblott and Gearhart

Application No.: 09/767,421

Filed: January 22, 2001

Page 3

9. (Currently amended). The <u>EBD celleells</u> of claim 1, wherein under suitable cell culture conditions the <u>EBD cells</u> proliferate for at least thirty population doublings without being immortal under said conditions.

PATENT

Attorney Docket No.: JHU1750-1

- 10. (Currently amended) The <u>EBD celleells</u> of claim [[1]] <u>9</u>, wherein under suitable cell culture conditions the <u>EBD cells</u> proliferate for at least sixty population doublings.
- 11. (Currently amended) The <u>EBD celleells</u> of claim 1, wherein the <u>EBD cells</u> proliferate under suitable cell culture conditions that are nonpermissive for proliferation of human embryonic germ cells.
- 12. (Currently amended) The <u>EBD celleells</u> of claim 1, wherein the <u>EBD cells</u> proliferate under suitable cell culture conditions lacking leukemia inhibitory factor, a fibroblast feeder layer, or both.
- 13. (Currently amended) The <u>EBD celleells</u> of claim 1, wherein the <u>EBD cells</u> are transfectable with a retrovirus or a lentivirus or both.
 - 14. (Canceled).
- 15. (Currently amended) The <u>EBD celleells</u> of claim [[1]] 9, wherein the <u>EBD cells</u> are clonal.
- 16. (Currently amended). The culture of claim 15, wherein the <u>EBD</u> cells are clonally derived from a single embryoid body derived (EBD) cell.
 - · 17-21. (Canceled).

In re Application of:
Shamblott and Gearhart

Application No.: 09/767,421 Filed: January 22, 2001

Page 4

PATENT Attorney Docket No.: JHU1750-1

- 22. (Currently amended) A method of obtaining a <u>human embryoid body derived (EBD)</u> <u>cellenriched population of cells</u> comprising:
- (a) culturing primordial germ cells under conditions that are suitable for formation of a solid or cystic embryoid bodies body having a 3-dimensional morphology;
- (b) <u>disaggregating dissociating</u>the <u>solid or cystic embryoid bodies body under suitable</u> <u>enzymatic conditions</u> to provide a constituent cell <u>or embryoid body derived (EBD) cell</u>; and
- (c) culturing the eonstituent <u>EBD</u> cell under conditions suitable to produce a population of <u>proliferating EBD</u> cells in serum, reduced serum or serum-free media and further comprising cells which simultaneously express a first and a second polypeptide or mRNA marker that is characteristic of at least two different cell types, wherein the cell types are selected from the group consisting of an ectodermal cell, a mesodermal cell, and an endodermal cell, and wherein the first marker is selected from the group consisting of nestin, vimentin, neurofilament light isoform, microtubule associated protein 2c, tau, nonphosphorylated neurofilament heavy isoform, neuron-specific enolase, tyrosine hydroxylase, glial fibrillary acidic protein, CNPase, and galactocerebroside and the second marker is selected from the group consisting of myf-6, myosin light-chain 2 ventricular isoform, flk-1, α-1-fetoprotein and GATA-4.
- 23. (Currently amended) The method of claim 22 comprising selecting a single <u>EBD</u> cell from the EBD cells culture and culturing the single EBD cell to produce a clonal population of cells.
- 24. (Currently amended) The method of claim 22 comprising culturing the constituent EBD cell in a media comprising human basic fibroblast growth factor.
- 25. (Currently amended) The method of claim 24 comprising culturing the constituent EBD cell in a media selected from the group consisting of RPMI 1640 supplemented with 15%

Shamblott and Gearhart

Application No.: 09/767,421 Filed: January 22, 2001

Page 5

Attorney Docket No.: JHU1750-1

PATENT

FCS and media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B, fetal bovine serum, VEGF, hFGF-2, heparin, recombinant human IGF-1 and ascorbic acid.

26. (Currently amended) The method of claim 25 comprising culturing the constituent EBD cell in a media consisting essentially of hEGF, hydrocortisone, gentamicin, amphotericin-B, fetal bovine serum, VEGF, hFGF-2, heparin, recombinant human IGF-1 and ascorbic acid.

- 27. (Currently amended) The method of claim 22 comprising culturing the constituent EBD cell on a matrix.
- 28. (Currently amended) The method of claim 27 comprising culturing the constituent EBD cell on a matrix that is selected from the group consisting of collagen I, human extracellular matrix extract, and tissue culture-treated plastic.
- 29. (Currently amended) The method of claim 28 comprising culturing the constituent EBD cell on a matrix selected from the group consisting of collagen I and human extracellular matrix.
- 30. (Currently amended) The method of claim 22 comprising culturing the constituent EBD cell on a media that is not permissive for proliferation of the EG cells.
- 31. (Currently amended) The method of claim 30 comprising culturing the constituent EBD cell on a media lacking leukemia inhibitory factor, a fibroblast feeder layer, or both.
- 32. (Currently amended) The method of claim 22 comprising culturing the population of proliferating EBD cells for at least 30 population doublings.

Shamblott and Gearhart

Application No.: 09/767,421 Filed: January 22, 2001

Page 6

- 33. (Canceled).
- 34. (New) The EBD cell of claim 1, wherein the enzyme includes collagenase, dispase, or both.

PATENT

Attorney Docket No.: JHU1750-1

- 35. (New) The method of obtaining a human EBD cell of claim 22, wherein the enzyme includes collagenase, dispase, or both.
- 36. (New) The method of claim 22, further comprising expanding the proliferating EBD cells on a matrix.
- 37. (New) The method of claim 36, wherein the matrix is selected from the group consisting of collagen I, human extracellular matrix extract, and tissue culture-treated plastic.
- 38. (New) A method of obtaining a human embryoid body derived (EBD) cell comprising:
- (a) culturing primordial germ cells under conditions that are suitable for formation of a solid or cystic embryoid body having a 3-dimensional morphology;
- (b) disaggregating the solid or cystic embryoid body under suitable enzymatic conditions to provide a constituent cell or embryoid body derived (EBD) cell; and
- (c) expanding the EBD cell under conditions suitable to produce a population of proliferating EBD cells, wherein the EBD cells proliferate on a matrix, and wherein the matrix is selected from the group consisting of collagen I, human extracellular matrix extract, and tissue culture-treated plastic.